REMARKS

In the final Office Action, the Examiner objected to claim 6; rejected claims 1, 3, 4, 6 and 8 under 35 U.S.C. § 102(b) as being anticipated by U.S. Patent No. 5,713,891 to Poppas; rejected claim 2 under 35 U.S.C. § 103(a) as being unpatentable over Poppas in view U.S. Patent No. 5,209,776 to Bass et al.; rejected claim 5 under 35 U.S.C. § 103(a) as being unpatentable over Poppas in view U.S. Patent No. 5,156,613 to Sawyer; and rejected claim 7 under 35 U.S.C. § 103(a) as being unpatentable over Poppas.

Applicants propose to amend claims 1 and 6. Upon entry of Applicants' proposed amendment, claims 1-8 will be pending in the above-captioned patent application.

By way of background, Applicants' disclosure is directed toward, among other things, a novel method for bonding first and second tissues with an adhesive and exposing the adhesive to electromagnetic radiation. In particular, tissues are joined together, the adhesive is applied, melted, and as it cools and solidifies, the tissues are bonded together. Tissue adhesives or solders for such applications must have sufficient mechanical properties to strongly join tissues in surgical applications (see Applicants' specification at page 1,

lines 8-9). Tissue adhesives should also be non-toxic (id., lines 10-14).

Applicants developed a novel composition that satisfies the above-described requirements of tissue adhesives, and is suitable for Applicants inventive tissue bonding method. The composition is collagen based, and is therefore non-toxic. Moreover, the high concentration of collagen provides a greater number of linkages so that upon exposure to laser light of a suitable wavelength, increased crosslinking with surrounding tissue can occur (specification at page 3, lines 1-3; page 4, lines 3-6). Accordingly, a tissue adhesive with improved cohesive strength can be achieved (Id.), and exceptional tensile strength of 1000g/cm$^2$. (specification at page 14, lines 19-20). By attaching the carboxyl group and carboxyl/thiol groups through derivatization, it is believed that the net negative charge of the adhesive ionically interacts with the positively charged proteins in tissues so that the adhesive is soluble at physiologic pH (page 9, lines page 8-11), so that the adhesive will dissolve within the body over time.

Applicants realized, however, that derivatized collagen solutions saturate at about 10% or 10 mg/ml (see page 10, lines 13-14), far short of the concentration

believed necessary to provide a sufficient number of cross-linking sites, as noted above. Exemplary prior art derivatized collagen concentrations limited to 10% are also described in U.S. Patent No. 6,183,498 (the '498 patent) at col. 8, lines 53-54 and col. 11, lines 4-6. The '498 patent is attached hereto for the Examiner's convenience.

In light of the limited solubility of known derivatized collagen-based solutions, Applicants developed a unique process to make their novel tissue adhesive. As described in the specification, derivatized collagen was successively added to a derivatized collagen solution, and the solution was heated to 50 degrees Celsius, for example (page 10, lines 18-20). With each addition, the concentration is increased until a desired concentration is achieved (page 10, lines 21-22). Heating the solution is believed to cause the derivatized collagen to break down into smaller molecular weight units. Accordingly, as further discussed in the specification, a gelatinized and derivatized collagen is obtained (page 10, line 18 - page 11, line 3).

Applicants note that amended claim 1 incorporates the subject matter of claim 6 related to carboxyl ($COO^-$) derivatization. Such subject matter has been redacted from claim 6, and claim 6 has been further amended to recite

collagen that is also derivatized with an $SH^-$ group. Proposed amended claim 1 also recites that the adhesive includes collagen that is gelatinized, support for which may be found in the specification (see, for example, page 10, line 18 - page 11, line 3). These claim changes are not deemed to raise new issues requiring further consideration and/or search.

Turning to the substance of the final Office Action, Applicants respectfully traverse the Examiner's objection to claim 6. The Examiner contends that "[d]erivatized is not a word." Applicants respectfully disagree.

The term "derivatized" is a commonly used term in protein chemistry to denote modification of a protein molecule. In fact, "derivatized" is mentioned in numerous instances in U.S. Patent Publication No. 2002/0098222 cited by the Examiner at page 2 of the final Office Action (see paragraphs 0036, 0042, 0043, 0044, 0045, 0122, 0132, 0246, in particular "[a]lbumin may be modified or derivatized ..." at paragraph 0042, and "[a]nother useful sealant formulation consists of collagen derivatized with glutaric anhydried and perfluorooctanoic acid (PFOA)" at paragraph 246. Applicants' use of the term "derivatized" in the specification (see e.g., page 10, lines 6-8) as well as claim 6 is consistent with such common usage. Accordingly,

Applicants respectfully request the Examiner to reconsider and withdraw the objection to claim 6.

Applicants respectfully traverse the Examiner's rejection of claims 1, 3, 4, 6, and 8 under 35 U.S.C. § 102(b) as being anticipated by Poppas. Amended claim 1, for example, is not anticipated by Poppas because the reference fails to teach each and every method step recited in the claim. In particular, Poppas at least fails to teach the step of providing an adhesive including collagen, whereby a concentration of said collagen in said adhesive being at least equal to 300 mg/ml, but less than 800 mg/ml. Moreover, Poppas fails to teach such collagen that is gelatinized and derivatized with a functional group selected from $COO^-$ and $SH^-$.

The Examiner acknowledges that Applicants' claimed concentration collagen is not disclosed in Poppas. Nevertheless, the Examiner contends that teachings of "50%" albumin compositions suggest that the claimed collagen concentration would be "inherent."

Applicants respectfully submit, however, that mere teachings of albumin concentrations fail to teach, render inherent, or otherwise suggest Applicants claimed concentration of collagen. Collagen, as generally understood, is much different protein than albumin, with

different properties and characteristics. Principal among them, the size of an albumin molecule, as indicated by is molecular weight, is only about 66,000 (see attached Sigma specification sheet), while the size of collagen is 300,00 (see attached web page of Worthington Biochemical Corporation). Since collagen molecules are relatively large, high concentration collagen based materials are more difficult to achieve than albumin based materials. Specifically, as indicated in Applicants specification, collagen concentrations in excess of 10%, (i.e., 100 mg/ml) are difficult to achieve (specification at page 10, lines 13-14). Such low concentrations are far short of the concentration believed necessary to provide a sufficient number of cross-linking sites, as noted above.

Applicants' claimed high collagen concentrations are not easily obtainable as noted in Applicants' specification, Applicants respectfully submit that the claimed concentrations would not have been apparent to those having ordinary skill and are certainly not inherent in the teachings of Poppas.

The Examiner relies on various references apparently in support of the assertion that Applicants' claimed collagen concentrations are known. Namely, the Examiner cites U.S. Patent No. 5,164,139 to Fujioka et al., U.S.

Patent 6,310,036 to Browdie, U.S. Published Application No. 2002/00225588 to Wilkie et al., and U.S. Published Application No. 2002/0098222 to Wironen et al. Applicants note that the Office Action fails to identify specific teachings in any of these references that corroborate the Examiner's positions, and submit that none of these references corroborates the Examiner's position.

Fujioka et al. discloses "collagen and/or gelatin (concentration : 10-50 w/w% ...)" (col. 3, lines 23-25). Browdie discloses a collagen concentration in a solution which is "between 35% to 45%" (col. 6, lines 16-17), and Wironen et al. is directed toward concentrations of gelatin (see paragraphs 49 and 52), formed by heating collagen for a sufficient period of time to effect "complete conversion to gelatin" (col. 4, lines 3-6). In contrast, Applicants claimed adhesive includes collagen that is both derivatized and gelatinized, but is not gelatin.

Further, none of the above references even discloses collagen that has been derivatized, particularly carboxyl derivatized collagen. Although Wilkie et al. describes derivatriziation of collagen with glutaric anhydride, the resulting concentration is only between 2 and 15 w/w%. As noted above, weight/weight concentrations are different than Applicants' claimed concentration in units of mg/ml.

Moreover, such teachings in Wilkie et al. are suggestive of concentrations substantially less than Applicants' claimed concentrations.

The Examiner also alleges that collagen "inherently have functional carboxyl groups with their associated conjugate acid-base pairs as explained by the well-known Brønsted-Lowry theory of acid-base reactions" (Office Action at page 2). Although non-derivatized collagen molecules may have a limited number of carboxyl groups, collagen also has a significant number of amine or $NH_3$ groups. As described in Applicants' specification, deriviatization with a carboxyl group involves substituting these amine groups with carboxyl groups (see specification at page 10, lines 6-8). Accordingly, collagen derivatized with a carboxyl group, as recited in amended claim 1, has substantially more carboxyl groups than naturally occurring collagen. The resulting collagen is thus soluble at physiologic pH (specification at page 9, lines 7-11), whereas non-derivatized collagen is only soluble in acidic pH.

As noted by the Examiner, Poppas discloses collagen, but is entirely silent as to whether such collagen is derivatized. As noted above, derivatization, a known term, denotes a modification of a protein molecule, and as

described in Applicants' specification involves substitution of amine groups with carboxyl or $COO^-$ functional groups. Such derivatized collagen, is different than the non-derivatized collagen described in Poppas, and is not inherently taught by Poppas.

In light of the above-described deficiencies of Poppas, Fujioka et al., Browdie, Wironen et al. and Wilkie et al., Applicants submit that amended claim 1 is allowable over the applied references. Moreover, claims 3 and 4 are allowable at least due to their dependence from claim 1.

Applicants respectfully traverse the Examiner's rejection of claim 2 under 35 U.S.C. § 103(a) as being unpatentable over Poppas in view to Bass et al.; the rejection of claim 5 under 35 U.S.C. § 103(a) as being unpatentable over Poppas in view Sawyer; and the rejection of claim 7 under 35 U.S.C. § 103(a) as being unpatentable over Poppas. In rejecting claim 2, the Examiner relies on Bass et al. allegedly for disclosing collagen welding and an infrared laser (see Office Action at page 3), and in rejecting claim 5, the Examiner contends that Sawyer teaches "use of a cyanoacrylate" (Office Action at page 3). The Examiner also asserts that the limitations of claim 7 are obvious over Poppas because "general conditions of [the] ... claim are disclosed in the prior art" (Office

Action at page 4). Applicants respectfully submit, however, that even if each of the Examiner contentions are correct, these teachings would nevertheless fail to overcome the above described shortcomings of Poppas. Accordingly, Applicants submit that claims 2, 5 and 7 are allowable at least due to their dependence from claim 1.

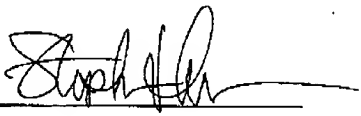
As note above, Applicants incorporation of limitations of claim 6, for example, into claim 1, and the changes to claim 6, do not raise new issues requiring further consideration and/or search. Applicants therefore respectfully request entry of their Amendment After Final, and a timely issuance of a Notice of Allowance.

If there are any other fees due in connection with the filing of this response, please charge the fees to our Deposit Account No. 020900.

If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

PTO is authorized to credit any overpayment to our Deposit Account.

Respectfully submitted,

By: 

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SIGMA®

# ProductInformation

**ALBUMIN, BOVINE**

**CAS NUMBER:** 9048-46-8

**SYNONYMS:** Bovine Serum Albumin; Bovine Plasma Albumin; BSA

**STRUCTURE:**

The molecular weight of BSA has frequently been cited as 66,120[1] or 66,267[2], but it was revised in 1990 to 66,430[3]. All three values are based on amino acid sequence information available at the time of publication.

BSA is a single polypeptide chain consisting of about 583 amino acid residues and no carbohydrates. At pH 5-7 it contains 17 intrachain disulfide bridges and 1 sulfhydryl group.[1,3]

**PHYSICAL PROPERTIES:**

Appearance: Powder - White to light tan[4];
Solutions - Clear to slightly hazy and amber[4]
pI in Water at 25°C: Endogenous Material[5,6,7] - 4.7; 4.9;
Fatty Acid Depleted[8] - 5.3
pH of 1% Solution:[1,4] 5.2-7;
Optical Rotation:[1,9] $[\alpha]_{259}$: -61°; $[\alpha]_{284}$: -63°
Stokes Radius ($r_s$):[10] 3.48 nm

| | |
|---|---|
| Sedimentation constant,[1] $S_{20,w}$ X $10^{13}$ | 4.5 (monomer), 6.7 (dimer) |
| Diffusion constant,[1] $D_{20,w}$ X $10^{7}$ | 5.9 |
| Partial specific volume,[1] $V_{20}$ | 0.733 |
| Intrinsic viscosity,[1] $\eta$ | 0.0413 |
| Frictional ratio,[1] $f/f_0$ | 1.30 |
| Overall dimensions,[1] Å | 40 X 140 |
| Refractive index increment[1] (578 nm) X $10^{-3}$ | 1.90 |
| Optical absorbance,[1] $A_{279nm}$ 1 gm/L | 0.667 |
| Mean residue rotation,[1] $[m]_{233}$ | 8443 |
| Mean residue ellipticity[1] | 21.1 $[\theta]_{209\ nm}$; 20.1 $[\theta]_{222\ nm}$ |
| Estimated α-helix,[1] % | 54 |
| Estimated β-form,[1] % | 18 |

**STABILITY / STORAGE AS SUPPLIED:**

If stored at 2-8°C, BSA powders and BSA solutions offered by Sigma are stable for a minimum of 2.5 years.[4]

## ALBUMIN, BOVINE

**SOLUBILITY / SOLUTION STABILITY:**

Albumins are readily soluble in water and can only be precipitated by high concentrations of neutral salts such as ammonium sulfate. Sigma tests the solubility of powdered BSA in deionized water at 40 mg/mL and obtains clear to very slightly hazy, faint yellow solutions. The solution stability of BSA is very good (especially if the solutions are stored as frozen aliquots). In fact, albumins are frequently used as stabilizers for other solubilized proteins (e.g., labile enzymes). However, albumin is readily coagulated by heat.[11] When heated to 50°C or above, albumin quite rapidly forms hydrophobic aggregates which do not revert to monomers upon cooling.[4] At somewhat lower temperatures aggregation is also expected to occur, but at relatively slower rates.

**METHOD OF PREPARATION:**

A. HISTORY:[1,4] Albumin is relatively simple to isolate and purify. One of the first methods of isolation involved extensive dialysis of serum against water; this process removed most globulins. A second procedure took advantage of the good solubility of albumin at low to moderate ammonium sulfate concentrations and effected precipitation by lowering the pH. Electrophoretic isolation was also employed, as was affinity chromatography. None of these methods were applicable to large scale production.

B. INITIAL ISOLATION: Initial isolation is by Heat Treatment or by Alcohol precipitation. Most commercial preparations are now prepared by Alcohol Precipitation a method developed by E. J. Cohn and his associates in the 1940's ("Fraction V" yields albumin with a purity of about 96%) or by Heat Treatment.[12]

C. FURTHER PURIFICATION:[1,4] Additional removal of impurities can be accomplished by crystallization (a procedure which yields ≥99% pure albumin), preparative electrophoresis, ion exchange chromatography, affinity chromatography (e.g., ConA-agarose removes glycoproteins), heat treatment (removes globulins), low pH treatment, charcoal treatment, organic solvent precipitation (i.e., isooctane), and low temperature treatment.[13] Charcoal treatment and organic solvent precipitation remove fatty acids.[13]

**PRODUCT DESCRIPTION / USAGE:**[14]

Albumins are a group of acidic proteins which occur plentifully in the body fluids and tissues of mammals and in some plant seeds. Unlike globulins, albumins have comparatively low molecular weights, are soluble in water, are easily crystallized, and contain an excess of acidic amino acids. Serum and plasma albumin is carbohydrate-free and comprises 55-62% of the protein present.

## ALBUMIN, BOVINE

**PRODUCT DESCRIPTION / USAGE:** (continued)

Albumin binds water, $Ca^{2+}$, $Na^{+}$, and $K^{+}$. Due to a hydrophobic cleft, albumin binds fatty acids, bilirubin, hormones and drugs. The main biological function of albumin is to regulate the colloidal osmotic pressure of blood. Human and bovine albumins contain 15% nitrogen and are often used as standards in protein calibration studies. Albumin is used to solubilize lipids, and is also used as a blocking agent in Western blots or ELISA applications. Globulin free albumins are suitable for use in applications where no other proteins should be present (e.g., electrophoresis).

**CHOOSING A PRODUCT:**

Please refer to the table below for a complete description of each product. Based on customer input, literature reports and Sigma's own use, the following table lists product numbers which have successfully been used for specific applications. The list is not comprehensive, and product numbers not listed may often be substituted.

## ALBUMIN, BOVINE

| APPLICATION | PRODUCT NUMBER(S) |
|---|---|
| Antibody purification | A-2058 |
| Binding and transport studies | A-4378, A-7030, A-0281, A-3675, A-3902, A-6003 |
| Blood banking reagents | A-2153, A-4503, A-7888, A-3294, A-3912, A-7906, A-7030 |
| Culture media (microbial) | A-2153, A-4503, A-3294, A-3912, A-7906, A-9430, A-7638, A-6003 |
| Cell culture (general) | A-8806, A-9418 |
| Electrophoresis (M.W. standard) | A-7517 |
| ELISA (blocking reagent) | A-2153, A-4503, A-4378, A-7030, A-9430, A-3902 |
| ELISA (non-specific binding) | A-3294 |
| Enzyme systems | A-2153, A-4503, A-7888, A-3294, A-3912, A-4378, A-7906, A-7030, A-9430, A-7638, A-3675 |
| Hapten carrier | A-7030, A-6003 |
| Immunocytochemistry | A-9647, A-7906, A-6793 |
| Immunohematology | A-2153, A-4503, A-7888, A-3294, A-3912, A-4378, A-7906, A-7030, A-0281, A-6003 |
| Mitogenic assays | A-2058 |
| Molecular biology | B-2518[15], B-8894[15], B-6917, B-8667, B-4287 |
| Protein base or filler | A-2153, A-4503, A-3912, A-4378, A-7906, A-7030 |
| Protein supplement (controls) | A-2153, A-4503, A-4378, A-7906, A-7030, A-3675 |
| Protein standard (M.W., amino acids, nitrogen) | A-2153, A-4503, A-4378, A-7030 |
| RIA systems | A-7888, A-4378, A-7030, A-3675, A-3902 |
| Serology | A-4503, A-3912, A-4378, A-7906, A-7030, A-9430, A-3675 |

## ALBUMIN, BOVINE

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15. These products are acetylated to inactivate nucleases commonly found in BSA, and are thus not listed in the table of unmodified BSA's on pp. 4-9 of this data sheet. Since tyrosines in the BSA are also derivatized, these preparations are not recommended for use as protein standards.

# Collagen

WBC Home
Manual/Protocol Index
Catalog Index

**Manual Page** | Catalog Page | Tissue Dissociation Guide

Collagen is an inert, rigid protein found predominantly in skin, ligaments, bones and teeth. Its most distinctive attribute, essential to a transmitter of mechanical force, is inelasticity. Its fundamental structural unit is tropo-collagen, a molecular rod about 2600 Å in length and 15 Å in diameter and 300,000 molecular weight. In tendons these macromolecules, grouped as collagen fibrils, run parallel to the axis, in skin the fibrils are interlaced and branched. Collagen has been reviewed by Gallop and Seifter (1963). See also the monograph on collagenase and review by Bornstein and Sage (1980).

Collagen fibers with limited crosslinkages (i.e. unaged) will dissolve to some extent in dilute acid or concentrated neutral salt solutions. Natural tendon (aged) collagen is insoluble in aqueous solutions.

Dissolved calf skin collagen in 0.075 M sodium citrate buffer, pH 4.3-4.5 (approx. 6 mg collagen/ml) can be repeatedly transformed into a stable gel on titration to pH 7.0 with 0.5 M sodium carbonate and by warming to 37°C. Such gels, cast as membranes or solids, are of interest; as for example, their use as support of immobilized enzymes. See Venkatasubramanian *et al.* (1974) and Wang and Vieth (1973).

Soluble collagen is also of importance in platelet aggregation assays (Swann *et al.*, 1974; Mustard *et al.*, 1973; Packman and Guccione, 1973; Puett and Cunningham, 1973; Jamieson *et al.*, 1971; and Nakanishi *et al.*, 1971). Worthington soluble calf skin collagen has been found to be suitable for this assay. It may be used directly or diluted with 0.9% saline.

**Storage**: Store at 2-8°C. Stable for many years if kept dry.

**Stability**: Bovine achilles tendon collagen is stable. Soluble calf skin collagen is stable for 3 - 6 months at 2 - 8°C.

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**References**

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